PATENT

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For: METHOD OF EXAMINING CHEMICAL :
USING GENE-DISRUPTED STRAIN :

SUBMISSION OF TRANSLATION

Assistant Commissioner of Patents
Washington, DC 20231

Sir:

Applicants submit herewith an English translation of International Patent Application No. PCT/JP2004/017779 including 70 pages and 6 sheets of drawing.

The attached document represents a true and complete English translation of International Patent Application No. PCT/JP2004/017779.

Respectfully submitted,

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DESCRIPTION

METHOD OF EXAMINING CHEMICAL USING GENE-DISRUPTED STRAIN

Technical field

[0001]

The present invention relates to a method of examining a chemical present in a specimen in the environment.

Background art

[0002]

A human being has previously produced a huge number of chemical substances, and new chemicals are developed every year. These chemicals are utilized in every aspect of a modern life, and serve in improving a life of a human being. To the contrary, among chemicals, some are released into the environment at a variety of stages such as manufacturing, distribution, use, disposal and the like, and adversely influence on health of a human and an ecosystem through remaining in the environment, and biological concentration due to a food chain, and environmental pollution has become a social problem. Therefore, there is demand for assessing influence of a chemical on a human body and an ecosystem.

When a chemical present in a test specimen to be detected, it is very important to improve a detection sensitivity of a detection system. When only a chemical having a low concentration is present in a test specimen, a test specimen must be concentrated depending on a detection sensitivity of a detection system which is used for detecting a chemical having a low concentration. However, in order to concentrate an aqueous solution such as an environmental specimen, a concentrating apparatus becomes necessary. In addition, when a subject chemical is volatile, a chemical is lost by a concentration procedure in some cases. For this reason, a detection system requiring necessity of concentrating procedure as little as possible, that is, an assay system having a high detection sensitivity is desired.

For detecting a chemical present in the environment, there is an assay system utilizing toxicity response of a yeast cell (Patent Publications 1 and 2).

[0003]

Patent Publication 1: WO 03/018792

Patent Publication 2: JP-A No. 2003-061676

Disclosure of the invention

Problems to be solved by the invention

[0004]

The present inventors accumulated gene information induced by chemicals as shown in Patent Publications 1 and 2, and have been studied a bioassay method utilizing toxicity response of a yeast cell. A sensitivity for detecting a chemical by bioassay depends on sensitivity of a cell and an organism using as an index on a chemical. Therefore, in a bioassay method utilizing toxicity response of a yeast cell, it is necessary to utilize a yeast cell having a higher sensitivity in order to construct a system of a higher sensitivity. Then, from about 4800 kinds of gene-disrupted strains which can be grown as a homozygous diploid among gene-disrupted strains of 6000 kinds of genes of yeast, gene-disrupted strains having a chemical sensitivity suitable in an assay system for detecting a chemical were selected.

An object of the present invention is to provide a method having a higher sensitivity in a bioassay method utilizing toxicity response of a microorganism.

Means to solve the problems

[0005]

That is, the present invention relates to:

(1) a method of examining whether a chemical is present in a test specimen or not, comprising culturing a gene-disrupted stain of a microorganism in the presence of the test specimen, and using cell response of the gene-disrupted strain to the chemical as an index, preferably the method in which cell response of the gene-disrupted strain to the chemical is life or death of a cell, and/or a change in the proliferating ability, an aspiration amount, enzyme activity and/or gene expression, further preferably the method in which the change in gene expression is a change in a RNA amount or a mRNA amount, more preferably the method in which the change in gene expression is measured by reporter·gene·assay,

[0006]

(2) the method according to the (1), wherein the microorganism is yeast, preferably the method in which a gene to be disrupted, according to classification of public database: MITS, is classified into amino acid metabolism (01.01), nitrogen and sulfur metabolism (01.02), nucleotide metabolism (01.03), phosphate metabolism (01.04), C-compound and carbohydrate metabolism (01.05), lipid, fatty acid and isoprenoid

metabolism (01.06), metabolism of vitamins, cofactors and prosthetic groups (01.07) of metabolism (01);

DNA processing (03.01), cell cycle (03.03) of cell cycle and DNA processing (03);

mRNA transcription (04.05), RNA transport (04.07) of transcription (04);

ribosome biosynthesis (05.01), translational control (05.07) of protein synthesis (05);

protein targeting, sorting, translocation (06.04), protein modification (06.07), assembly of protein complex (06.10), proteolysis (06.13) of protein fate (06);

nuclear transport (08.01), vesicular transport (Golgi network etc.) (08.07), vacuolar transport (08.13), cellular import (08.19), cytoskeleton-dependent transport (08.22), other intracellular transport activities (08.99) of intracellular transport and transport mechanism (08);

stress response (11.01), toxicification (11.07) of cell rescue, defense and pathogenicity (11);

ionic homeostasis (13.01), cell sensitivity and response (13.11) of intracellular environmental regulation/interaction (13);

cell growth/morphogenesis (14.01), cell differentiation (14.04) of cell fate (14);

cell wall (30.01), cytoskeleton (30.04), nucleus (30.10), mitochondria (30.16) of cell tissue control (30);

ion transporter (67.04), vitamin/cofactor transporter (67.21), transport mechanism (67.50), other transport promotion (67.99) of transport promotion (67);

unclassified (98); and/or

unclassified protein (99), further preferably the method in which the gene to be disrupted is involved in the function of the following Table 2, more preferably, the method in which the gene to be disrupted is involved in a vacuole, for example, in the case of yeast, specifically, the following YPR036W, YDR027C, YHR026W, YHR039C-A, YKL080W, YLR447C, YGR105W, YKL119C, YHR060W (wherein YHR039C-A is designated as YHR039C-B in some cases),

[0007]

more specifically, the method in which the gene to be disrupted is

(2-1) YGL026C, YGR180C, YDR127W, YCR028C, YLR284C, YOR221C, YAL021C, YGL224C, YBL042C, YDR148C, YHL025W, YLR307W, YLR345W, YLR354C, YPL129W or YPR060C which is a metabolism (01) gene;

(2-2) YGR180C, YDR150W, YGL240W, YBL058W, YIL036W, YLR226W, YLR381W, YOR026W, YPL018W, YBL063W, YDR363W-A, YIR026C, YLR234W, YMR032W or YPL129W which is a cell cycle and DNA processing (03) gene;

(2-3) YGR006W, YIL036W, YKR082W, YLR226W, YML112W, YMR021C, YAL021C, YDR195W, YOL068C, YBR279W, YGL070C,

YGL071W, YGL222C, YHL025W, YLR266C or YPL129W which is a transcription (04) gene;

(2-4) YBL058W, YLR287C-A, YGR084C or YLR344W which is a protein synthesis (05) gene;

(2-5) YKL080W, YLR447C, YGL240W, YGR105W, YGL206C, YKL119C, YDR414C, YHR060W, YLR292C, YLR306W, YGL227W or YGR270W which is a protein fete (06) gene;

(2-6) YPR036W, YDR027C, YHR039C, YKL080W, YLR447C, YGL206C, YKR082W, YLR292C or YBL063W which is an intracellular transport and transport mechanism (08) gene;

(2-7) YJR104C or YMR021C which is a detoxification (11) gene;

(2-8) YPR036W, YHR039C, YKL080W, YLR447C, YGL071W or YIR026C which is an intracellular regulation/interaction (13) gene;

(2-9) YDL151C, YBL058W, YKR082W, YDL151C, YOL068C, YDR363W-A, YHL025W, YIR026C, YLR307W, YMR032W or YPL129W which is a cell fate (14) gene;

(2-10) YDR027C, YDR414C, YLR381W, YGR084C or YMR032W which is cell tissue control (30) gene;

(2-11) YPR036W, YHR026W, YHR039C, YKL080W, YLR447C, YCR028C or YLR292C which is a transport promotion (67) gene;

(2-12) YBL056W which is an unclassified (98) gene; or

(2-13) YDR149C, YLR285W, YLR311C, YOR331C, YPR123C,

YDR525W-A, YDR539W, YDR540C, YGL246C, YJL204C, YLR282C, YLR287C, YLR290C, YJL188C, YJL192C, YJL211C, YKL037W, YLR283W, YLR312C, YLR315W, YLR320W or YPL030W which is an unclassified (99) gene;

[0008]

(3) the method according to the (1), wherein the microorganism is a microorganism other than yeast, and the gene to be disrupted is a gene corresponding to a gene as defined in the (2),

[0009]

(4) a kit comprising a gene-disrupted strain of a microorganism, which is used for examining whether a chemical is present in a test specimen or not, preferably,

the kit, wherein cell response to a chemical is life or death of a cell, and/or a change in the proliferating ability, aspiration amount, enzyme activity and/or gene expression, further preferably,

the kit, wherein the change in gene expression is a change in a RNA amount or a mRNA amount, more preferably,

the kit, wherein the change in gene expression is measured by reporter·gene·assay,

(5) the kit according to the (4), wherein the microorganism is yeast and the gene to be disrupted is defined in the (2),

and the kit according to the (4), wherein the microorganism is a microorganism other than yeast, and the gene to be disrupted is a gene corresponding to a gene as defined in the (2),

[0010]

(6) a composition for examining whether a chemical is present in a test specimen or not, comprising a gene-disrupted strain of a microorganism, preferably,

the composition, wherein cell response to a chemical is life or death of a cell, and/or a change in the proliferating ability, an aspiration amount, enzyme activity and /or gene expression, further preferably,

the composition, wherein the change in gene expression is a change in a RNA amount or a mRNA amount, more preferably, the composition, wherein the change in gene expression is measured by reporter·gene·assay,

(7) the composition according to the (6), wherein the microorganism is a microorganism other than yeast, and the gene to be disrupted is defined in the (2), and the composition according to the (6), wherein the microorganism is a microorganism other than yeast, and the gene to be disrupted is a gene corresponding to a gene as defined in the (2), and

(8) use of a gene-disrupted strain of a microorganism for

examining whether a chemical is present in a test specimen or not, preferably,

the use, wherein cell response to a chemical is life or death of cell a and/or a change in the proliferating ability, an aspiration amount, enzyme activity and/or gene expression, further preferably,

the use, wherein the change in gene expression is a change in a RNA amount or a mRNA amount, more preferably,

the use, wherein the change in gene expression is measured by reporter·gene·assay,

(9) the use according to the (8), wherein the microorganism is a microorganism other than yeast, and the gene to be disrupted is defined in (2), and the use according to the (8), wherein the microorganism is a microorganism other than yeast, and the gene to be disrupted is a gene corresponding to a gene as defined in the (2).

Effect of the invention

[0011]

The present invention is a highly sensitive assay system which can suitably detect a chemical even when only a chemical having a low concentration is present in a test specimen. Since the assay system of the present invention has a high sensitivity, it is not necessary to concentrate a test specimen and, since concentration is not necessary,

even when a subject chemical is volatile, a chemical can be suitably detected.

Brief description of the drawings

[0012]

[Fig. 1] Fig. 1 is a graph showing a sensitivity to sodium metaarsenite in a gene-disrupted strain DEL011 transformed with a plasmid p-YPL171C.

[Fig. 2] Fig. 2 is a graph showing a sensitivity to sodium metaarsenite in gene-disrupted stains DEL011, DEL014 and DEL016 transformed with a plasmid p-YBR072W.

[Fig. 3] Fig. 3 is a graph showing a sensitivity to cadmium chloride in gene-disrupted strains DEL002, DEL010, DEL016, DEL019 and DEL025 transformed with a plasmid p-YBR072W.

[Fig. 4] Fig. 4 is a graph showing a sensitivity to bentiocarb in gene-disrupted strains DEL000, DEL019, DEL022 and DEL025 transformed with a plasmid p-YBR072W.

[Fig. 5] Fig. 5 is a graph showing a sensitivity to mercuric chloride in gene-disrupted strains DEL011, DEL016 and DEL025 transformed with a plasmid p-YPL171C.

[Fig. 6] Fig. 6 is a graph showing a sensitivity to sodium metaarsenite in gene-disrupted strains DEL006 and DEL014 transformed with a plasmid p-YPL171C at a concentration which is 1/30 a concentration of a gene-non-disrupted strain, and in DEL003, DEL008 and DEL022 at a concentration which is 1/3 a concentration of a gene-non-disrupted strain. All of gene-disrupted strains are a homozygous diploid.

[Fig. 7] Fig. 7 is a graph showing a sensitivity to sodium metaarsenite in a homozygous diploid of a gene-disrupted stain DEL014 transformed with a plasmid p-YPL171C, and in a heterozygous diploid DEL000/014 at a concentration which is 1/30 a concentration of a gene-non-disrupted strain.

[Fig. 8] Fig. 8 is a graph showing a sensitivity to thiuram in gene-disrupted stains DEL007 and DEL022 transformed with a plasmid p-YPL171C at a concentration which is 1/1000 a concentration of a gene-non-disrupted strain, and in DEL001 and DEL0020 at a concentration which is 1/3 a concentration of a gene-non-disrupted stain. All of gene-disrupted strains are a homozygous diploid.

[Fig. 9] Fig. 9 is a graph showing a sensitivity to thiuram in a homozygous diploid and a heterozygous diploid of a gene-disrupted strain DEL006 transformed with a plasmid p-

YPL171C at a concentration which is 1/10 a concentration of a gene-non-disrupted strain.

[Fig. 10] Fig. 10 is a graph showing a sensitivity to benthiocarb in gene-disrupted strains DEL006, EL007 and DEL022 transformed with a plasmid p-YBR072W at a concentration which is 1/10 a concentration of a gene-non-disrupted strain, and in DEL012, DEL013 and DEL020 at a concentration which is 1/3 a concentration of a gene-non-disrupted strain. All of gene-disrupted strains are a homozygous diploid.

[Fig. 11] Fig. 11 is a graph showing a sensitivity to benthiocarb in a homozygous diploid of a gene-disrupted strain DEL0022 transformed with a plasmid p-YBR072W at a concentration which is 1/10 a concentration of a gene-non-disrupted strain, and in a heterozygous diploid of a gene-disrupted strain DEL0022 at a concentration which is 1/3 a concentration of a gene-non-disrupted strain.

Best mode for carrying out the invention

[0013]

One aspect of the present invention will be explained by referring to a yeast gene.

1) Selection of gene-disrupted strain and classification of function thereof

Among 4800 kinds of gene-disrupted strains of Yeast Deletion Homozygous Diploid (YKO Plate sets: Yeast Deletion Homozygous Diploid complete set, ResGen™, Invitrogen) used as a yeast gene-disrupted strain, 84 kinds of strains showing a better sensitivity to a chemical were selected (Example 1). Disrupted genes of 84 kinds of strains were classified according to classification of public database: MIPS (Munich Information center for Protein Sequences). Classification of MIPS classifies genes based on functions thereof, and the information can be easily obtained from the following URL: http://mips.gsf.de/genre/proj/yeast/searchCatalogFirstAction.do?style=catalog.xslt&table=FUNCTIONAL CATEGORIES

According to classification of MIPS, yeast genes are classified as shown in the following Table:

Table 1

[Table 1-1]

01 .. Metabolism
01.01 .. Amino acid metabolism
01.02 .. Nitrogen and sulfur metabolism
01.03 .. Nucleotide metabolism
01.04 .. Phosphate metabolism
01.05 .. C-compound and carbohydrate metabolism
01.06 .. Lipid, fatty acid and isoprenoid metabolism
01.07 .. Metabolism of vitamins, cofactors and prosthetic groups
01.20 .. Secondary metabolism
02 .. Energy

02.01 .. Glycolysis and Gluconeogenesis
02.07 .. Pentose-phosphate pathway
02.10 .. Tricarboxylic-acid pathway (citrate cycle, Krebs cycle, TCA cycle)
02.11 .. Electron transport and membrane-associated energy conservation
02.13 .. Respiration
02.16 .. Fermentation
02.19 .. Energy storage metabolism (e.g. glycogen, trehalose)
02.22 .. Glyoxylic acid cycle
02.25 .. Oxidation of fatty acid
02.99 .. Other energy generation activities
03 .. Cell cycle and DNA processing
03.01 .. DNA processing
03.03 ..Cell cycle
03.99 .. Other cell division and DNA synthesis activities
04 .. Transcription
04.01 .. rRNA transcription
04.03 .. tRNA transcription
04.05 .. mRNA transcription
04.07 .. RNA transport
04.99 .. Other transcription activities
05 .. Protein synthesis
05.01 .. Ribosome biosynthesis
05.04 .. Translation
05.07 .. Translational control
05.10 .. Aminoacyl-tRNA-synthases
05.99 .. Other protein synthesis activities

[Table1-2]

06 .. Protein fate (folding, modification, destination)
06.01 .. Protein folding and stabilization
06.04 .. Protein targeting, sorting and translocation
06.07 .. Protein modification
06.10 .. Assembly of protein complexes
06.13 .. Proteolysis
06.99 .. Other protein fate-associated activities
08 .. Intracellular transport and transport mechanism
08.01 .. Nuclear transport
08.04 .. Mitochondrial transport
08.07 .. Vesicular transport (Golgi network etc.)
08.10 .. Peroxisomal transport
08.13 .. Vacuolar transport
08.16 .. Extracellular transport, exocytosis and secretion
08.19 .. Cellullar import
08.22 .. Cytoskeleton-dependent transport
08.99 .. Other intracellular transport activities
10 .. Cell transmission/signal transmitting mechanism
10.01 .. Intracellular signaling
10.05 .. Transmembrane signal transmission
11 .. Cell rescue, defense and pathogenicity
11.01 .. Stress response

11.07 .. Detoxification
11.10 .. Degradation of foreign compounds
11.99 .. Other cell rescue activities
13 .. Intracellular environmental regulation interaction
13.01 .. Ionic homeostasis
13.11 .. Cell sensitivity and response
14 .. Cell fate
14.01 .. Cell growth/morphogenesis
14.04 .. Cell differentiation
14.10 .. Cell death
14.20 .. Cell aging

[Table1-3]

29 .. Transpositional element, virus and plasmid protein
29.07 .. Protein necessary for integrating or inhibiting transposon transfer
29.99 .. Other transpositional element, virus and plasmid protein
30 .. Cell tissue control
30.01 .. Cell wall
30.02 .. Plasma membrane
30.03 .. Cytoplasm
30.04 .. Cytoskeleton
30.05 .. Centrorsome
30.07 .. Endoplasmic reticulum
30.08 .. Golgi
30.09 .. Intracellular transport vesicle
30.10 .. Nucleus
30.16 .. Mitochondria
30.19 .. Peroxisome
30.22 .. Endosome
30.25 .. Vacuole and lysosome
30.99 .. Other control of cell tissue
40 .. Intracellular sorting
40.01 .. Cell wall
40.02 .. Plasma membrane
40.03 .. Cytoplasm
40.04 .. Cytoskeleton
40.05 .. Centrosome
40.07 .. Endoplasmic reticulum
40.08 .. Golgi
40.09 .. Intracellular transport vesicle
40.10 .. Nucleus
40.16 .. Mitochondria
40.19 .. Peroxisome
40.22 .. Endosome
40.25 .. Vacuole and lysosome
40.27 .. Extracellular/secretion protein

[Table 1-4]

62 .. Protein activity regulation
62.01 .. Regulation mechanism
62.02 .. Regulation target
63 .. Element necessary for protein or cofactor having binding function (structural or catalytic)
63.01 .. Protein binding
63.03 .. Nucleic acid binding
63.09 .. Lipid binding
67 .. Transport promotion
67.01 .. Channel/pore class transporter
67.04 .. Ion transporter
67.07 .. C-compound and carbohydrate transporter
67.10 .. Amino acid transporter
67.11 .. Peptide transporter
67.13 .. Lipid transporter
67.16 .. Nucleotide transporter
67.19 .. Allantoin and allantoate transporter
67.21 .. Vitamin/cofactor transporter
67.28 .. Drug transporter
67.50 ..Transport mechanism
67.99 .. Other transport promotion
98 .. Unclassified
99 .. Unclassified protein

[0014]

Eighty four kinds of selected strains exhibiting better sensitivity to a chemical were classified according to the aforementioned database: MIPS classification.

Table 2.

Classification based on function

Chemical sensitivity Functional classification of genes of 84 gene-disrupted strains

[Table 2-1]

Function	No	Gene	MIPS classification	description
METABOLISM 01	DEL003	YGL026C	01.01.01	Tryptophan synthase
	~~DEL004~~	~~YGR180C~~	~~01.03.07~~	Ribonucleotide reductase small

				subunit
	DEL009	YDR127W	01.01.01	Arom pentafunctional enzyme
	DEL016	YCR028C	01.02.04 01.05.04 01.06.10 01.07.10	Pantothenate permease
	DEL023	YLR284C	01.06.04	Delta3-cis-delta2-trans-enoyl-CoA isomerase
	DEL028	YOR221C	01.06.07	Malonyl-CoA:ACP transferase
	DEL031	YAL021C	01.05.04	Transcriptional regulator
	DEL038	YGL224C	01.03.04	Pyrimidine 5-nucleotidase
	DEL052	YBL042C	01.03.04	Uridine permease
	DEL056	YDR148C	01.05.01	2-Oxoglutarate dehydrogenase complex E2 component
	DEL064	YHL025W	01.05.04	Global transcription activator
	DEL073	YLR307W	01.05.01	Sporulation-specific chitin deacetylase
	DEL078	YLR345W	01.05.04	Similarity to Pfk26p and other 6-phosphofructo-2-kinases
	DEL079	YLR354C	01.05.01	Transaldolase

[Table 2-2]

	DEL082	YPL129W	01.04.04 01.05.04	TFIIFsubunit (transcription initiation factor), 30 kD
	DEL083	YPR060C	01.01.01	chorismate mutase
CELL CYCLE AND DNA PROCESSING 03	DEL004	YGR180C	03.01.03	ribonucleotide reductase small subunit
	DEL010	YDR150W	03.03.01	nuclear migration protein
	DEL011	YGL240W	03.03.01	component of the anaphase promoting complex
	DEL015	YBL058W	03.03.01 03.03.02	potential regulatory subunit for Glc7p
	DEL019	YIL036W	03.01.03	ATF/CREB activator
	DEL022	YLR226W	03.03.01	divergent CDK-cyclin complex
	DEL048	YLR381W	03.03.04 .05	outer kinetochore protein
	DEL050	YOR026W	03.03.01	cell cycle arrest protein
	DEL051	YPL018W	03.03.04 .05	outer kinetochore protein
	DEL054	YBL063W	03.03.01	kinesin-related protein
	DEL057	YDR363W-A	03.03.01	regulator of exocytosis and pseudohyphal differentiation
	DEL065	YIR026C	03.03.02	Protein tyrosine phosphatase
	DEL070	YLR234W	03.03.01	DNA topoisomerase III
	DEL080	YMR032W	03.03.03	involved in cytokinesis

[Table 2-3]

	DEL082	YPL129W	03.03.01	TFIIF subunit (transcription initiation factor), 30 kD

TRANSCRIPTION 04	DEL012	YGR006W	04.05.05 .01	U5 snRNA-associated protein
	DEL019	YIL036W	04.05.01 .04	ATF/CREB activator
	DEL021	YKR082W	04.07	nuclear pore protein
	DEL022	YLR226W	04.05.01 .04	divergent CDK-cyclin complex
	DEL026	YML112W	04.05.01 .04	carboxy-terminal domain (CTD) kinase, gamma subunit
	DEL027	YMR021C	04.05.01 .04	metal binding activator
	DEL031	YAL021C	04.05.01 .04	transcriptional regulator
	DEL033	YDR195W	04.05.05	RNA 3^-end formation protein
	DEL049	YOL068C	04.05.01 .04	silencing protein
	DEL055	YBR279W	04.05.01 .04	DNA-directed RNA polymerase II regulator
	DEL058	YGL070C	04.05.01 .01	DNA-directed RNA polymerase II, 14.2 KD subunit
	DEL059	YGL071W	04.05.01 .04	iron-regulated transcriptional repressor
	DEL060	YGL222C	04.05.05 .03	stimulates mRNA decapping
	DEL064	YHL025W	04.05.01 .04	global transcription activator
	DEL071	YLR266C	04.05.01 .04	weak similarity to transcription factors

[Table 2-4]

	DEL082	YPL129W	04.05.01 .01	TFIIF subunit (transcription initiation factor), 30 kD
PROTEIN SYNTHESIS 05	DEL015	YBL058W	05.07	potential regulatory subunit for Glc7p
	DEL044	YLR287C -A	05.01	40S small subunit ribosomal protein
	DEL062	YGR084C	05.01	mitochondrial ribosomal protein, small subunit
	DEL077	YLR344W	05.01	60S large subunit ribosomal protein
PROTEIN FATE (folding, modification, destination) 06	DEL007	YKL080W	06.10	H+-ATPase V1 domain 42 KD subunit, vacuolar
	DEL008	YLR447C	06.10	H+-ATPase V0 domain 36 KD subunit, vacuolar
	DEL011	YGL240W	06.07 06.13.01	component of the anaphase promoting complex
	DEL013	YGR105W	06.10	ATPase assembly integral membrane protein
	DEL018	YGL206C	06.04	clathrin heavy chain
	DEL020	YKL119C	06.10	H+-ATPase assembly protein
	DEL034	YDR414C	06.04 06.07	Putative transport protein of inner membranes

[Table 2-5]

	DEL040	YHR060W	06.10	vacuolar ATPase assembly protein
	DEL046	YLR292C	06.04	ER protein-translocation complex subunit
	DEL047	YLR306W	06.07	E2 ubiquitin-conjugating enzyme
	DEL061	YGL227W	06.13.04	weak similarity to human RANBPM NP_005484.1
	DEL063	YGR270W	06.13.01	26S proteasome subunit
CELLULAR TRANSPORT AND TRANSPORT MECHANISMS 08	DEL000	YPR036W	08.13	H+-ATPase V1 domain 54 KD subunit, vacuolar
	DEL002	YDR027C	08.07	subunit of VP51-54 complex, required for protein sorting at the yeast late Golgi
	DEL006	YHR039C-A	08.13	H+-transporting ATPase V0 domain 13 KD subunit, vacuolar
	DEL007	YKL080W	08.13	+-ATPase V1 domain 42 KD subunit, vacuolar
	DEL008	YLR447C	08.13	H+-ATPase V0 domain 36 KD subunit, vacuolar
	DEL018	YGL206C	08.19	clathrin heavy chain
	DEL021	YKR082W	08.01	nuclear pore protein
	DEL046	YLR292C	08.99	ER protein-translocation complex subunit
	DEL054	YBL063W	08.22	kinesin-related protein

[Table 2-6]

11.07 .. detoxification	DEL014	YJR104C	11.07	copper-zinc superoxide dismutase
	DEL027	YMR021C	11.01	metal binding activator
13 .. REGULATION OF / INTERACTION WITH CELLULAR ENVIRONMENT	DEL000	YPR036W	13.01.01.03	H+-ATPase V1 domain 54 KD subunit, vacuolar
	DEL006	YHR039C-A	13.01.01.03	H+-transporting ATPase V0 domain 13 KD subunit, vacuolar
	DEL007	YKL080W	13.01.01.01	H+-ATPase V1 domain 42 KD subunit, vacuolar
	DEL008	YLR447C	13.01.01.03	H+-ATPase V0 domain 36 KD subunit, vacuolar
	DEL059	YGL071W	13.01.01.01	iron-regulated transcriptional repressor
	DEL065	YIR026C	13.11.03.01	protein tyrosine phosphatase
14 .. CELL	DEL001	YDL151C	14.04.03.01	involved in bipolar bud site selection

FATE	DEL015	YBL058W	14.04.03.01	potential regulatory subunit for Glc7p
	DEL021	YKR082W	14.04.03.05	potential regulatory subunit for Glc7p
	DEL032	YDL151C	14.04.03.01	involved in bipolar bud site selection
	DEL049	YOL068C	14.04.03.03	silencing protein
	DEL057	YDR363W-A	14.04.03.01	regulator of exocytosis and pseudohyphal differentiation

[Table 2-7]

	DEL064	YHL025W	14.04.03.03	global transcription activator
	DEL065	YIR026C	14.04.03.05	protein tyrosine phosphatase
	DEL073	YLR307W	14.04.03.05	sporulation-specific chitin deacetylase
	DEL080	YMR032W	14.01 14.04.03.01	involved in cytokinesis
	DEL082	YPL129W	14.04.03.03	30 kD:TFIIF subunit (transcription initiation factor), 30 kD
30 .. CONTROL OF CELLULAR ORGANIZATION	DEL002	YDR027C	30.01 30.04.03	subunit of VP51-54 complex, required for protein sorting at the yeast late Golgi
	DEL034	YDR414C	30.01	Putative transport protein of inner membranes
	DEL048	YLR381W	30.10.03	outer kinetochore protein
	DEL062	YGR084C	30.16	mitochondrial ribosomal protein, small subunit
	DEL080	YMR032W	30.04	involved in cytokinesis
67.. TRANSPORT FACILITATION	DEL000	YPR036W	67.04.01.02 67.50.22	H+-ATPase V1 domain 54 KD subunit, vacuolar
	DEL005	YHR026W	67.04.01.02 67.50.22	H+-ATPase 23 KD subunit, vacuolar
	DEL006	YHR039C-A	67.04.01.02 67.50.22	H+-transporting ATPase V0 domain 13 KD subunit, vacuolar
	DEL007	YKL080W	67.04.01.02 67.50.22	H+-ATPase V1 domain 42 KD subunit, vacuolar

[Table 2-8]

	DEL008	YLR447C	67.04.01.02 67.50.22	H+-ATPase V0 domain 36 KD subunit, vacuolar
	DEL016	YCR028C	67.21	Pantothenate permease
	DEL046	YLR292C	67.99	ER protein-translocation complex subunit
UNCLASSIFIED PROTEINS	DEL053	YBL056W	98.	ser/thr protein phosphatase PP2C
	DEL017	YDR149C	99.	
	DEL024	YLR285W	99.	weak similarity to A.thaliana hypothetical protein
	DEL025	YLR311C	99.	weak similarity to S.tarentolae cryptogene protein G4
	DEL029	YOR331C	99.	
	DEL030	YPR123C	99.	
	DEL035	YDR525W-A	99.	PMP3/SNA1(similarity)
	DEL036	YDR539W	99.	similarity to E.coli hypothetical 55.3 kDa protein in rfah-rfe intergenic region
	DEL037	YDR540C	99.	similarity to E. coli unknown gene
	DEL039	YGL246C	99.	weak similarity to C.elegans dom-3 protein

[Table 2-9]

	DEL041	YJL204C	99.	involved in recycling of the SNARE Snc1p
	DEL042	YLR282C	99.	
	DEL043	YLR287C	99.	weak similarity to S.pombe hypothetical protein SPAC22E12
	DEL045	YLR290C	99.	similarity to hypothetical protein SPCC1840.09 S. pombe
	DEL066	YJL188C	99.	
	DEL067	YJL192C	99.	facilitates ER export of the yeast plasma membrane [H+]ATPase, Pma1
	DEL068	YJL211C	99.	
	DEL069	YKL037W	99.	weak similarity to C.elegans ubc-2 protein
	DEL072	YLR283W	99.	weak similarity to Smc2p
	DEL074	YLR312C	99.	hypothetical protein
	DEL075	YLR315W	99.	weak similarity to rat apolipoprotein A-IV
	DEL076	YLR320W	99.	hypothetical protein

	DEL08 1	YPL030 W	99.	similarity to C.elegans hypothetical protein

[0015]

Further, gene-disrupted strains exhibiting sensitivity to 7 or more kinds of chemicals among 12 kinds of chemicals which were tested in the following Examples are classified based on function, as in Table 3.

[0016]

Table 3. Classification depending on function

[Table 3]

Function	Number of gene-disrupted strains
Metabolism-amino acid metabolism(01.01)	2
Metabolism-C-compound and carbohydrate metabolism(01.05)	1
Lipid, fatty acid and isoprenoid metabolism(01.06)	3
Cell cycle and DNA processing-DNA processing(03.01)	2
Cell cycle and DNA processing-cell cycle(03.03)	4
Transcription-mRNA transcription(04.05)	5
Protein fate (folding, modification, destination)-protein modification(06.07)	1
Protein fate (folding, modification, destination)-protein complex assembling(06.10)	4
Intracellular transport and transport mechanism-vacuolar transport(08.13)	3
Intracellular environmental regulation/interaction-ionic homeostasis (13.01)	3
Cell fate-cell differentiation(14.04)	3
Transport promotion-ion transporter(67.04)	4
Transport promotion-transport mechanism(67.50)	4
Unclassified protein(99)	4

[0017]

When the same gene has overlapped functions, it was counted repeatedly. Particularly, there were many overlaps in intracellular transport and transport mechanism-vacuolar transport (08.13), intracellular environmental regulation/interaction-ionic homeostasis (13.01), transport promotion-ion transporter (67.04), and transport promotion-transport regulation (67.50).

In particular, genes are overlapped in intracellular transport and transport mechanism-vacuole transport (08.13), intracellular environmental regulation/interaction-ionic homeostasis (13.01), transport promotion-ion transporter (67.04), and transport promotion-transport mechanism (67.50) and, since 50% of higher 10 genes were in this category, it was confirmed by this study that a vacuole plays an important role in detoxificating a chemical. In addition, it was seen that transcription-mRNA transcription (04.05), cell cycle and DNA synthesis-cell cycle (03.03), cell fate-cell differentiation (14.04), cell cycle and DNA synthesis-DNA synthesis (03.01), protein fate (folding, modification, destination)-protein complex assembling (06.10), metabolism-amino acid biosynthesis (01.01), metabolism-C-bond, carbohydrate metabolism (01.05), lipid, fatty acid, isoprenoid metabolism (01.06) are also involved in response to a chemical. Further, usefulness of genes

whose functions were not known was confirmed.

[0018]

In the present invention, a microorganism other than yeast can be used. Herein, as a microorganism, any of an animal cell derived from human, mouse and other mammal, and an established strain of an animal cell, and cells of fishes, a nematode and the like, an insect cell, a eukaryote cell such as yeast and the like, and a bacterial cell such as Escherichia coli may be used. And, when a gene-disrupted strain of a gene corresponding to a gene having function found in the yeast utilizing known database is made by the known procedure, it can be utilized in the method of the present invention. Particularly, genes corresponding to function described as "description" in classification based on function in Table 2 can be utilized as a subject of a disrupted gene in a disrupted strain.

[0019]

(2)Use of selected gene-disrupted strains

By destructing a particular gene, a microorganism exhibits sensitivity or resistance to a chemical in some cases.

In the present invention, the "gene-disrupted strain" includes a monoploid gene-disrupted strain, a homozygous

diploid gene-disrupted strain and a heterozygous diploid gene-disrupted strain. A yeast cell can form a diploid by mating between an α-type cell and an a-type cell which are a monoploid. A homozygous diploid gene-disrupted strain is a strain in which genes disrupted in α and a are the same and, on the other hand, a heterozygous diploid gene-disrupted strain refers to a strain in which a gene disrupted in α and a gene disrupted in a are different, and a strain in which only a gene in α or a is disrupted. The number of genes to be disrupted is not limited to one, but a plurality of genes among those listed above may be disrupted.

[0020]

In the present invention, a gene-disrupted strain having an improved sensitivity to a chemical is selected, and utilized for assaying a chemical. The presence of a chemical is assayed utilizing, as an index, cell response to a chemical of a gene-disrupted strain. Cell response to a chemical shows life or death of a cell, and/or proliferation ability an aspiration amount, enzyme activity and/or a change in gene expression.

Herein, "life or death of a cell" can be measured and assessed by a ratio of a living cell or an ATP amount,

"proliferation ability" by a ratio of increase in a cell number, "aspiration amount" by a consumed amount of oxygen, "enzyme activity" by enzyme activity originally possessed by an index cell and "change in gene expression" by a RNA amount or a mRNA amount. In addition, in the present invention, as measurement of a change in particular gene expression, a method of measuring an expression amount of a particular gene measured by a Northern blotting method (Molecular Biology of Cell, second edition, published by Kyouiku-sha Co., Ltd. in 1990, pp.189-191) or an reporter·gene·assay method can be also utilized.

Among them, a method of measuring life or death of a cell, proliferation ability, an aspiration amount, or a change in expression of a particular gene is a simple procedure and suitable in bioassay. The reporter·gene·assay is procedure of measuring activity of a particular gene as a mark for investigating function of a gene laying stress on transcription activity, and includes a promoter assay method. The promoter assay method is a method of ligating operatively a polynucleotide encoding a marker protein to the polynucleotide sequence of a promoter of a gene and indirectly measuring expression of a gene (Barelle CJ, Manson CL, MacCallum DM, Odds FC, Gow Na, Brown AJ. :GFP as a quantitative reporter of gene regulation in Candida albicans. Yeast 2004 Mar; 21(4):333-40).

[0021]

A gene-disrupted strain which can be suitably used in chemical detection in the present invention using cell response as an index includes the following strains in which a gene is disrupted:

YPR036W, YDL151C, YDR027C, YGL026C, YGR180C, YHR026W, YHR039C-A, YKL080W, YLR447C, YDR127W, YDR150W, YGL240W, YGR006W, YGR105W, YJR104C, YBL058W, YCR028C, YDR149C, YGL206C, YIL036W, YKL119C, YKR082W, YLR226W, YLR284C, YLR285W, YLR311C, YML112W, YMR021C, YOR221C, YOR331C, YPR123C, YAL021C, YDL151C, YDR195W, YDR414C, YDR525W-A, YDR539W, YDR540C, YGL224C, YGL246C, YHR060W, YJL204C, YLR282C, YLR287C, YLR287C-A, YLR290C, YLR292C, YLR306W, YLR381W, YOL068C, YOR026W, YPL018W, YBL042C, YBL056W, YBL063W, YBR279W, YDR148C, YDR363W-A, YGL070C, YGL071W, YGL222C, YGL227W, YGR084C, YGR270W, YHL025W, YIR026C, YJL188C, YJL192C, YJL211C, YKL037W, YLR234W, YLR266C, YLR283W, YLR307W, YLR312C, YLR315W, YLR320W, YLR344W, YLR345W, YLR354C, YMR032W, YPL030W, YPL129W and YPR060C.

[0022]

When a change in gene expression is selected as cell response to a chemical and the gene change is measured by reporter · gene · assay, plasmids which can be utilized in

reporter gene assay are described in WO03/01872. In one aspect of the present invention, a plasmid containing a polynucleotide in which a polynucleotide encoding a marker protein is operatively connected to a polynucleotide sequence containing a promoter of a yeast gene described in WO 03/01872 is utilized.

[0023]

Preferable combinations of a gene-disrupted strain which can be suitably used, and a chemical which can be detected are as follows:

Table 4.

Correspondence of gene disrupted strain and chemical

[Table 4-1]

Disrupted gene	Number of chemical	Kind of Chemical
YPR036W	10	methylmercury chloride, sodium arsenite, nickelous chloride, a potassium dichromate triphenyltin=chloride, mercuric chloride, lead chloride, SDS-DMSO, zinc chloride
YDL151C	9	sodium arsenite, nickelous chloride, potassium dichlomate, triphenyltin=chloride, mercuric chloride, lead chloride, SDS DMSO, zinc chloride
YDR027C	9	sodium arsenite, nickelous chloride, triphenyltin=chloride, mercuric chloride, lead chloride, SDS,DMSO, zinc chloride
YGL026C	9	sodium arsenite, nickelous chloride, triphenyltin= chloride, mercuric chloride, copper sulfate,lead chloride, SDS,DMSO, zinc chloride
YGR180C	9	methylmercury chloride, sodium arsenite, potassium dichromate triphenyltin=chloride, mercuric chloride, lead chloride, SDS, DMSO, zinc chloride
YHR026W	9	methylmercury chloride, sodium arsenite,

		nickelous chloride, potassium dichromate triphenyltin=chloride, mercuric chloride, lead chloride, DMSO, zinc chloride
YHR039C-A	9	methylmercury chloride, sodium arsenite, potassium dichromate, triphenyltin=chloride, mercuric chloride, lead chloride, SDS, DMSO, zinc chloride
YKL080W	9	methylmercury chloride, sodium arsenite, nickelous chloride, a triphenyltin=chloride, mercuric chloride, lead chloride, SDS, DMSO, zinc chloride
YLR447C	9	sodium arsenite, nickelous chloride, potassium dichromate, triphenyltin= chloride, mercuric chloride, lead chloride, SDS, DMSO, zinc chloride
YDR127W	8	nickelous chloride, triphenyltin= chloride, mercuric chloride, copper sulfate, lead chloride, SDS, DMSO, zinc chloride
YDR150W	8	methylmercury chloride, sodium arsenite, potassium dichromate, trilphenyltin = chloride, mercuric chloride, copper sulfate, potassium cyanide, zinc chloride
YGL240W	8	methylmercury chloride, triphenyltin=chloride, mercuric chloride, coppersulfate, potassium cyanide, SDS DMSO, zinc chloride
YGR006W	8	methylmercury chloride, triphenyltin=chloride, mercuric chloride, coppersulfate, potassium cyanide, lead chloride, SDS, zinc chloride
YGR105W	8	nickelous chloride, potassium dichromate, triphenyltin= chloride, mercuric chloride, lead chloride, SDS, DMSO, zinc chloride
YJR104C	8	methylmercury chloride, soium arsenite, potassium dichromate chloride, a triphenyltin= chloride, mercuric chloride, SDS DMSO, zinc chloride
YBL058W	7	sodium arsenite, triphenyltin= chloride, mercuric chloride, copper sulfate, lead chloride, DMSO, zinc chloride
YCR028C	7	methylmercury chloride, triphenyltin=chloride, mercuric chloride, copper sulfate, SDS, DMSO, zinc chloride
YDR149C	7	methylmercury chloride, sodium arsenite, potassium dichromate, mercuric chloride, potassium cyanide, lead chloride, zinc chloride
YGL206C	7	sodium arsenite, nickelous chloride, potassium dichromate, mercuric chloride, lead chloride, SDS, DMSO

[Table 4-2]

YIL036W	7	methylmercury chloride, sodium arsenite, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, zinc chloride
YKL119C	7	sodium arsenite, potassium dichromate,

		triphenyltin=chloride, mercuric chloride, lead chloride, SDS, zinc chloride
YKR082W	7	potassium dichromate, triphenyltin=chloride, mercuric chloride, potassium cyanide, lead chloride, DMSO, zinc chloride
YLR226W	7	methylmercury chloride, potassium dichromate, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, zinc chloride
YLR284C	7	triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, SDS, DMSO, zinc chloride
YLR285W	7	methylmercury chloride, triphenyltin=chloride, coppersulfate, lead chloride, SDS, DMSO, zinc chloride
YLR311C	7	methylmercury chloride, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, DMSO, zinc chloride
YML112W	7	methylmercury chloride, sodium arsenite, nickelous chloride, potassium dichromate, triphenyltin=chloride, mercuric chloride, DMSO
YMR021C	7	methylmercury chloride, sodium arsenite, triphenyltin=chloride, mercuric chloride, SDS, DMSO, zinc chloride
YOR221C	7	methylmercury chloride, sodium arsenite, mercuric chloride, coper sulfate, lead chloride, DMSO, zinc chloride
YOR331C	7	nickelous chloride, potassium dichromate, triphenyltin=chloride, mercuric chloride, lead chloride , SDS, zinc chloride
YPR123C	7	methylmercury chloride, sodium arsenite, nickelous chloride, triphenyltin=chloride, mercuric chloride, DMSO, zinc chloride
YAL021C	6	sodium arsenite, potassium dichromate, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride
YDL151C	6	methylmercury chloride, sodium arsenite, mercuric chloride, copper sulfate, lead chloride, SDS
YDR195W	6	sodium arsenite, potassium dichromate, triphenyltin=chloride, mercuric chloride, potassium cyanide, DMSO
YDR414C	6	potassium dichromate, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, zinc chloride
YDR525W-A	6	triphenyltin=chloride, copper sulfate, lead chloride, SDS, DMSO, zinc chloride
YDR539W	6	triphenyltin=chloride, copper sulfate, lead chloride, SDS, DMSO, zinc chloride
YDR540C	6	triphenyltin=chloride, mercuric chloride, copper sulfate, SDS, DMSO, zinc chloride
YGL224C	6	methylmercury chloride, triphenyltin=chloride, copper sulfate, potassium cyanide, lead chloride, zinc chloride
YGL246C	6	methylmercury chloride, triphenyltin=chloride, lead chloride, SDS, DMSO, zinc chloride
YHR060W	6	methylmercury chloride, triphenyltin=chloride, mercuric chloride, lead chloride, DMSO, zinc

		chloride
YJL204C	6	triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, DMSO, zinc chloride
YLR282C	6	triphenyltin=chloride, mercuric chloride, lead chloride, ,SDS, DMSO, zinc chloride
YLR287C	6	triphenyltin=chloride, mercuric chloride, copper sulfate, ,SDS, DMSO, zinc chloride

[Table 4-3]

YLR287C-A	6	triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, SDS, DMSO
YLR290C	6	triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, SDS, zinc chloride
YLR292C	6	mercuric chloride, copper sulfate, lead chloride, SDS, DMSO, zinc chloride
YLR306W	6	methylmercury chloride, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, zinc chloride
YLR381W	6	triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, DMSO, zinc chloride
YOL068C	6	methylmercury chloride, potassium dichromate, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride
YOR026W	6	nickelous chloride, triphenyltin=chloride, mercuric chloride, lead chloride, SDS, DMSO
YPL018W	6	triphenyltin=chloride, mercuric chloride, copper sulfate, potassium cyanide, lead chloride, DMSO
YBL042C	5	mercuric chloride, copper sulfate, lead chloride, DMSO, zinc chloride
YBL056W	5	potassium dichromate, copper sulfate, lead chloride, DMSO, zinc chloride
YBL063W	5	triphenyltin=chloride, mercuric chloride, lead chloride, DMSO, zinc chloride
YBR279W	5	methylmercury chloride, potassium dichromate, mercuric chloride, SDS, DMSO
YDR148C	5	potassium dichromate, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride
YDR363W-A	5	triphenyltin=chloride, copper sulfate, lead chloride, DMSO, zinc chloride
YGL070C	5	triphenyltin=chloride, mercuric chloride, potassium cyanide, SDS, DMSO
YGL071W	5	nickelous chloride, potassium dichromate, triphenyltin=chloride, mercuric chloride, zinc chloride
YGL222C	5	methylmercury chloride, sodium arsenite, triphenyltin=chloride, copper sulfate, zinc chloride
YGL227W	5	mercuric chloride, copper sulfate, potassium cyanide, lead chloride, zinc chloride
YGR084C	5	copper sulfate, lead chloride, SDS, DMSO, zinc chloride
YGR270W	5	sodium arsenite, potassium dichromate, mercuric chloride, copper sulfate, zinc chloride
YHL025W	5	sodium arsenite, potassium

		dichromate, ,triphenyltin=chloride, mercuric chloride, DMSO
YIR026C	5	sodium arsenite ,triphenyltin=chloride, lead chloride, SDS, zinc chloride
YJL188C	5	mercuric chloride, copper sulfate, lead chloride, DMSO, zinc chloride
YJL192C	5	triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, DMSO
YJL211C	5	methylmercury chloride, triphenyltin=chloride, copper sulfate, DMSO, zinc chloride
YKL037W	5	sodium arsenite, triphenyltin=chloride, mercuric chloride, DMSO, zinc chloride

[Table 4-4]

YLR234W	5	nickelous chloride, mercuric chloride, lead chloride, SDS, DMSO
YLR266C	5	nickelous chloride, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride
YLR283W	5	copper sulfate, lead chloride, SDS, DMSO, zinc chloride
YLR307W	5	triphenyltin=chloride, mercuric chloride, lead chloride, DMSO, zinc chloride
YLR312C	5	triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride, zinc chloride
YLR315W	5	methylmercury chloride, sodium arsenite, potassium dichromate, triphenyltin=chloride, potassium cyanide
YLR320W	5	sodium arsenite, potassium dichromate, triphenyltin=chloride, potassium cyanide, zinc chloride
YLR344W	5	mercuric chloride, copper sulfate, SDS, DMSO, zinc chloride
YLR345W	5	copper sulfate, lead chloride, SDS, DMSO, zinc chloride
YLR354C	5	mercuric chloride, lead chloride, SDS, DMSO, zinc chloride
YMR032W	5	potassium dichromate, triphenyltin=chloride, mercuric chloride, copper sulfate, lead chloride
YPL030W	5	triphenyltin=chloride, mercuric chloride, copper sulfate, potassium cyanide, SDS
YPL129W	5	methylmercury chloride, potassium dichromate, triphenyltin=chloride, lead chloride, zinc chloride
YPR060C	5	nickelous chloride, mercuric chloride, lead chloride, SDS, DMSO

[0024]

(3) Kit

A kit of the present invention contains a container

containing a dried product, for example, a lyophilized product, a L-dried product or a frozen product of the gene-disrupted strain, a culturing medium and the like.

As the culturing medium, a medium having a suitable composition for a gene-disrupted strain to be used, is used.

[0025]

(4) Composition

As another aspect, the present invention provides a composition containing a gene-disrupted strain of a microorganism for detecting whether a chemical is present in a test specimen or not. Typically, a present composition is the culturing medium containing the gene-disrupted strain.

Examples

The present invention will be explained in more detailed below by Examples, but the present invention is not limited to these Examples.

Example 1

Test of chemical sensitivity of gene-disrupted strain using growth inhibition in chemical plate as index.

a)Method

As a yeast gene-disrupted strain, Yeast Deletion Homozygous Diploid (YKO Plate sets: Yeast Deletion Homozygous Diploid complete set, ResGen™, Invitrogen) was used. A parent strain of this gene-disrupted strain is Saccharomyces crevisiae BY4743. Among 6000 kinds of yeast gene- disrupted strains, a plurality of disrupted strains which can be chemical-sensitive are selected. Some of actual gene-disrupted strains can not be grown depending on a gene when it is defective. Then, as subject of the present experiment, about 4800 kinds of gene-disrupted strains which can be grown as Homozygous diploids were selected.

[0027]

The frozen and stored gene-disrupted strain was grown to the steady state by shaking-culturing at 25 °C on a YPD medium (yeast extract 1%, polypeptone 2%, glucose 2%). Cells in the steady state were diluted 10000-fold with the same medium, and each 1.5μL of diluted cells were added dropwise to a chemical-containing agar medium (Chemical Plate), and formation of colonies was observed after three days. Chemical plate was made by adding a chemical to a YPD agar medium (yeast extract 1%, polypeptone 2%, glucose 2%, agar 2%) to a final concentration shown in Table 5.

[0028]

Table 5. Chemicals in sensitivity experiment of gene-disrupted strain by chemical plate

[Table 5]

No	Chemical	Concentration		
C001P	Methylmercury chloride	0.07 μM	0.2 μM	0.6 μM
C002P	Sodium arsenite	0.3 mM	1 mM	3 mM
C003P	Nickelous chloride	1 mM	3 mM	9 mM
C004P	Potassium dichromate	0.3 mM	1 mM	3 mM
C005P	Triphenyltin=chloride	0.007 mM	0.02 mM	0.06 mM
C006P	Mercuric chloride	0.033 mM	0.1 mM	0.3 mM
C007P	Copper sulfate	2.67 mM	8 mM	24 mM
C008P	Potassium cyanide	6 mM	18 mM	54 mM
C009P	Lead chloride	0.67 mM	2 mM	6 mM
C010P	SDS	0.003%	0.01%	0.03%
C011P	DMSO	1%	3%	9%
C012P	Zinc chloride	3.3 mM	10 mM	30 mM

[0029]

b) Results

Experiment of chemical sensitivity was performed for about 4800 kinds of gene-disrupted strains. From test results, the number of chemicals to which sensitivity was exhibited was calculated for each gene-disrupted strain, and summarized in Table 6. Herein, exhibiting sensitivity refers to growth inhibition of a parent strain at two or more concentrations. Two or more concentrations means that when growth was compared at different three concentrations for each chemical as shown in Table 5, growth is worse, or growth is not seen at two or more concentrations as

compared with growth of a parent strain. For growth of a cell, life or death of a cell, and proliferation ability (growth number or growing rate) were used as an index.

Table 6. Number of gene-disrupted strains exhibiting sensitivity to chemical

[Table 6]

Number of chemicals to which sensitivity was exhibited	Number of gene-disrupted strains
0	4149
1	348
2	135
3	59
4	61
5	32
6	21
7	16
8	6
9	8
10	1
11	0
12	0

[0030]

Among about 4800 of gene-disrupted strains, the number of gene-disrupted strains exhibiting sensitivity to 10 kinds of chemicals is 1, the number is 8 to 9 kinds of chemicals, 6 to 8 kinds of chemicals, 16 to 7 kinds of chemicals, 21 to 6 kinds of chemicals, 32 to 5 kinds of chemicals, 61 to 4 kinds of chemicals, 59 to 3 kinds of chemicals, 135 to 2 kinds of chemicals, 348 to 1 kind of chemical, and the number of strains exhibiting no

sensitivity to chemicals was 4149. Particularly, gene-disrupted strains exhibiting sensitivity to 5 or more chemicals are shown in Table 7.

[0031]

Gene-disrupted strains exhibiting sensitivity to 5 or more chemicals

[Table 7-1]

Name of disrupted strain	Disrupted gene	Number of chemicals exhibiting growth inhibition at 2 or more concentrations
DEL000	YPR036W	10
DEL001	YDL151C	9
DEL002	YDR027C	9
DEL003	YGL026C	9
DEL004	YGR180C	9
DEL005	YHR026W	9
DEL006	YHR039C-A	9
DEL007	YKL080W	9
DEL008	YLR447C	9
DEL009	YDR127W	8
DEL010	YDR150W	8
DEL011	YGL240W	8
DEL012	YGR006W	8
DEL013	YGR105W	8
DEL014	YJR104C	8
DEL015	YBL058W	7
DEL016	YCR028C	7
DEL017	YDR149C	7
DEL018	YGL206C	7
DEL019	YIL036W	7
DEL020	YKL119C	7
DEL021	YKR082W	7
DEL022	YLR226W	7
DEL023	YLR284C	7
DEL024	YLR285W	7
DEL025	YLR311C	7
DEL026	YML112W	7
DEL027	YMR021C	7
DEL028	YOR221C	7
DEL029	YOR331C	7
DEL030	YPR123C	7

DEL031	YAL021C	6
DEL032	YDL151C	6
DEL033	YDR195W	6
DEL034	YDR414C	6
DEL035	YDR525W-A	6
DEL036	YDR539W	6
DEL037	YDR540C	6
DEL038	YGL224C	6
DEL039	YGL246C	6
DEL040	YHR060W	6

[Table 7-2]

DEL041	YJL204C	6
DEL042	YLR282C	6
DEL043	YLR287C	6
DEL044	YLR287C-A	6
DEL045	YLR290C	6
DEL046	YLR292C	6
DEL047	YLR306W	6
DEL048	YLR381W	6
DEL049	YOL068C	6
DEL050	YOR026W	6
DEL051	YPL018W	6
DEL052	YBL042C	5
DEL053	YBL056W	5
DEL054	YBL063W	5
DEL055	YBR279W	5
DEL056	YDR148C	5
DEL057	YDR363W-A	5
DEL058	YGL070C	5
DEL059	YGL071W	5
DEL060	YGL222C	5
DEL061	YGL227W	5
DEL062	YGR084C	5
DEL063	YGR270W	5
DEL064	YHL025W	5
DEL065	YIR026C	5
DEL066	YJL188C	5
DEL067	YJL192C	5
DEL068	YJL211C	5
DEL069	YKL037W	5
DEL070	YLR234W	5
DEL071	YLR266C	5
DEL072	YLR283W	5
DEL073	YLR307W	5
DEL074	YLR312C	5
DEL075	YLR315W	5
DEL076	YLR320W	5
DEL077	YLR344W	5
DEL078	YLR345W	5
DEL079	YLR354C	5

DEL080	YMR032W	5
DEL081	YPL030W	5
DEL082	YPL129W	5
DEL083	YPR060C	5

[0032]

Example 2

Study of detection sensitivity of homozygous diploid gene-disrupted strain using promoter assay

As described above, when a detectable sensitivity is low, generally, pre-treatment such as concentration of a sample and the like becomes necessary and, in particular, when concentration is performed at a high rate, there is a possibility that a chemical as a subject is lost during a concentration procedure. A detection sensitivity of a chemical by a reporter · gene · assay method depends on sensitivity of an index organism. As a method of increasing sensitivity without changing an index organism, it is contemplated that a line having high sensitivity is selected among the same species. It is thought that, there is a possibility that sensitivity is improved due to various reasons by lost of a gene, such as increase in membrane permeability of a chemical due to lost of a gene of a constitutional component of a cell membrane, and response to a chemical at a low concentration due to lost of a gene involved in detoxification mechanism and, herein, as a line exhibiting a different nature, an attention is

paid to a gene-disrupted strain. How a chemical damages an organism, and how an organism responses thereto has not previously been analyzed comprehensively. Then, by selecting a gene-disrupted strain exhibiting sensitivity to many kinds of chemicals by experiment, the gene-disrupted strain may be used as an index organism. There are about 6000 genes in a yeast cell, and since strains with a deleted gene have already been made and sold regarding almost all genes, screening was performed using them.

Method

1) Selection of gene-disrupted strain

In a gene-disrupted strain, a growing rate becomes small so much, or medium components in which the strain can be grown are different in some cases, depending on a disrupted gene. Then, in the present experiment studying a host cell of a promoter assay method, in view of easy comparison with a control experiment, among gene-disrupted strains obtained as the result of Example 1, a few strains which have sensitivity to many chemicals and are grown by the same procedure as that of a parent strain were selected. Selected gene-disrupted strains are 8 strains of DEL000, DEL002, DEL011, DEL014, DEL016, DEL019, DEL022 and DEL025 in Table 7. Further, as a control, a parent strain, BY4743 was used.

[0033]

2) Preparation of transformant

A competent cell of each of a parent strain of a gene-disrupted strain and selected gene-disrupted strains was prepared. This competent cell was transformed using two kinds of prepared plasmids for promoter assay, p-YBR072W (in which GFP was connected understream of a promoter of YBR072W) and p-YPL171C (in which GFP was connected downstream of a promoter of YPL171C). YPL171C is a gene encoding NAPDH dehydrogenase, YBR072W is a gene encoding a heat shock protein, and both of them exhibit response to a plurality of kinds of chemicals when prompter assay is performed.

Specifically, p-YBR072W was prepared by the following procedure. Primers for amplifying a polynucleotide (SCPD: disclosed in the Promoter Database of Saccharomyces cerevisiae) (SEQ ID No:1) containing a promoter sequence of a yeast gene YBR072W by PCT were prepared. Primers were designed using Oligo 4.0-S, Sequencher I, a McIntosh version, which is a software for designing primers, a nucleotide sequence of an upper primer is:

GCAGTCAACGAGGAGCGAATCAG (SEQ ID NO: 2),

and a nucleotide sequence of a lower primer is:

GTTAATTTGTTTAGTTTGTTTG (SEQ ID NO:3)

In PCR, as a template, a yeast chromosome (Saccharomyces cerevisiae S288C, Cat.40802, Reserch Genetics, Inc.) was used and, as a reagent a commercially available kit (KOD DNA Polymerase; code KOD-101, Toyobo) was used.

As a vector, pYES2 (pYES2, Cat no:V825-20, Invitrogen Corporation, USA) (R.W.OLD, S.B. Primrose Principle of Gene Manipulation, Original Document, 5th Edition, BaifuKan Co., Ltd., pp.234-263, 2000)) as a YEp-type shuttle vector which is replicated in both of Escherichia coli and yeast was used. As a polynucleotide encoding a marker protein, GFP, a part (SEQ ID NO: 4) of GFP of a vector pQBI 63 (Cat no.54-0082, Wako Pure Chemical Industries Ltd.) was used. First, a vector in which a polynucleotide of GFP was inserted into a multiple cloning site of pYES2 was made. Then, a part of a GAL promoter pYES2 was replaced with a polynucleotide containing a promoter sequence of YBR072W which is a yeast gene, to obtain an objective plasmid vector. A procedure of insertion of a polynucleotide containing GFP and a promoter sequence was performed by selecting appropriate restriction enzymes.

[0034]

Then, yeast Saccharomyces cerevisiae BY4743 (YKO Plate sets: Yeast Deletion Homozygous Diploid complete set, ResGen™, Invitrogen) was transformed with this plasmid vector. A procedure of transformation is shown below.

1) A yeast cell, Saccharomyces cerevisiae BY4743 is shaking-cultured on 200 mL of a YPD medium until OD660 becomes 0.5.

2) Cells are collected and suspended in 5 mL of a TE-buffer

3) 250 µL of 2.5 M lithium acetate is added.

4) Each 300 µL is dispended, and 10 µL of the plasmid vector is added, followed by culturing at 30°C for 30 minutes.

5) 700 µL of 50% PEG4000 is added, followed by shaking-culturing at 30°C for 60 minutes.

6) After heat shock (42°C, 5 minutes), the culture is rapidly cooled.

7) The culture is washed with 1 M sorbitol twice.

8) This is seeded on an agar plate made of a minimum nutrient medium (obtained by adding a necessary amino acid (histidine, leucine) to a SD medium).

[0035]

Transformation was confirmed on a selective medium(SD medium (Yeast nitrogen base without amino acids (Difco 0919-15)+glucose+amino acid (histidine, leucine). For

colonies which were grown an agar plate of the selective medium were further confirmed for amino acid auxotrophy.

[0036]

And, p-YPL171C was prepared as follows:

Primers for amplifying a polynucleotide (SCPD: disclosed in The Promoter Database of Saccharomyces cerevisiae) (SEQ ID No. 5) containing a promoter sequence of a yeast gene YPL171C by PCR was prepared. Primers were designed using Oligo 4.0-S, Sequencher I, a McIntosh version, which is a software for designing primers, a nucleotide sequence of an upper primer is:

ACGCCCCTTCCTTTTTCCCTTTC (SEQ ID No: 6)

and a nucleotide sequence of a lower primer is:

CTTCTAAATTTAAACTTCGCTA (SEQ ID No: 7)

In PCR, as a template, a yeast chromosome (Saccharomyces cerevisiae S288C, Cat.40802, Reserch Genetics, Inc.) was used and, as a reagent, a commercially available kit (KOD DNA Polymerase; code KOD-101, Toyobo) was used.

As a vector, pYES2 (pYES2, Cat no:V825-20, Invitrogen Corporation, USA) (R.W. Old, S.B. Primrose, Principle of

Gene Manipulation, original document 5th edition, Baifukan Co., Ltd., pp.234-263, 2000) as a YEp-type shuttle vector which is replicated in both of Escherichia coli and yeast was used. In addition, as a polynucleotide encoding a marker protein GFP, a part (SEQ ID No: 4) of GFP of a vector pQBI 63 (Cat no.54-0082, Wako Pure Chemical Industries Ltd.) was used. First, a vector in which a polynucleotide of GFP was inserted into a multiple cloning site of pYES2 was prepared. Then, a part of a GAL1 promoter of pYES2 was replaced with a polynucleotide containing a prompter sequence of YPL171C which is a yeast gene, to obtain an objective plasmid vector. A procedure for inserting a polynucleotide containing GFP and a promoter sequence was performed by selecting appropriate restriction enzymes.

[0037]

Then, a yeast Saccharomyces cerevisiae BY4743 (YKO Plate sets: Yeast Deletion Homozygous Diploid complete set, ResGen™, Invitrogen) was transformed with this plasmid vector. A procedure of transformation is shown below.

1) A yeast cell, Saccharomyces cerevisiae BY4743 is shaking-cultured on 200 mL of a YPD medium until OD660 becomes 0.5.

2) Cells are collected and suspended in 5 mL of a TE-buffer

3) 250 μL of 2.5 M lithium acetate is added.

4) Each 300 μL is dispended, and 10 μL of the plasmid vector is added, followed by culturing at 30°C for 30 minutes.

5) 700 μL of 50% PEG4000 is added, followed by shaking-culturing at 30°C for 60 minutes.

6) After heat shock (42°C, 5 minutes), the culture is rapidly cooled.

7) The culture is washed with 1 M sorbitol twice.

8) This is seeded on an agar plate made of a minimum nutrient medium (obtained by adding a necessary amino acid (histidine, leucine) to a SD medium).

[0038]

Transformation was confirmed on a selective medium (SD medium (Yeast nitrogen base without amino acids (Difco 0919-15)+glucose+amino acid (histidine, leucine). For colonies which were grown an agar plate of the selective medium were further confirmed for amino acid auxotrophy.

[0039]

3) Chemical sensitivity test

The resulting transformant was grown to the steady state by shaking-culturing on a SD medium (histidine, leucine) at 25°C. The transformant in the steady state was

diluted 500-fold with the same medium, shaking-cultured at 25°C for 15 hours and, after it was confirmed that an absorbance at 600nm was 0.2 to 0.5 as a logarithsmic growth phase, chemicals having different concentrations were loaded. After loading of chemicals, fluorescence of cells which had been cultured for 4 hours was measured using a flow cytometer (FITC filter, EPICS XL-MCL, Bechmancoulter), and this was adopted as an expression amount of GFP (green fluorescence protein) which is a marker gene. A fluorescence intensity of 10000 cells was measured with a flow cytometer by one measurement and an average of fluorescence intensities of all cells was obtained, and was adopted as a measured value. Similarly, a fluorescence intensity of a cell to which a chemical had not been loaded was obtained, and results are shown as a fluorescence intensity ratio.

[0040]

4) Results

A detection sensitivity of a promoter assay method when gene-disrupted strains DEL000, DEL002, DEL011, DEL014, DEL016, DEL019, DEL022, and DEL025 (Table 7) were used as a host cell, was studied. As a chemical to be loaded, sodium metaarsenite, cadmium chloride, benthiocarb and mercury (II) chloride which exhibit response when BY4743 was a host,

were selected and used. A dilution series of a chemical was made, a loading test was performed and results are shown in Figs 1 to 5.

Fig. 1 shows that a gene-disrupted strain DEL011 responded to sodium metaarsenite at a concentration which is 1/3 a concentration of a parent strain.

Fig. 2 shows that a gene-disrupted strain DEL011 responded to sodium metaarsenite at a concentration which is 1/10 a concentration of a parent strain, a gene-disrupted strain DEL014 at a concentration which is 1/3000 a concentration of a parent strain, and a gene-disrupted strain DEL016 at a concentration which is 1/3 a concentration of a parent strain.

Fig. 3 shows that a gene-disrupted strain DEL002 responded to cadmium chloride at a concentration which is 1/3 a concentration of a parent strain, a gene-disrupted strain DEL011 at a concentration which is 1/3 a concentration of a parent strain, DEL016 at a concentration which is 1/3, DEL019025 at a concentration which is 1/3, and a gene-disrupted strain DEL at a concentration which is 1/3 a concentration of a parent strain.

Fig. 4 shows that a gene-disrupted strain DEL000 responded to benthiocarb at a concentration which is 1/3 a concentration of a parent strain, a gene-disrupted strain DEL 019 at a concentration which is 1/100 a concentration of a parent strain, DEL022 at a concentration which is 1/10, and a gene-disrupted strain DEL025 at a concentration which is 1/3.

Fig. 5 shows that a gene-disrupted strain DEL011 responded to mercuric chloride at a concentration which is 1/10 a concentration of a parent strain, and a gene-disrupted strain DEL016 at a concentration which is 1/3.

Like this, it was confirmed that DEL000, DEL002, DEL011, DEL014, DEL016, DEL019, DEL022 and DEL025 have responsiveness to a chemical which is 3-fold to 100-fold higher than that of a parent strain, BY4743. Particularly, even at a concentration which is 1/1000 a detectable concentration of a parent strain, a significant difference was seen in DEL0014, as compared with BY4743 (Fig 2).

[0041]

Example 3

Study of detection sensitivity of homozygous and heterozygous diploid gene-disrupted strains using promoter

assay

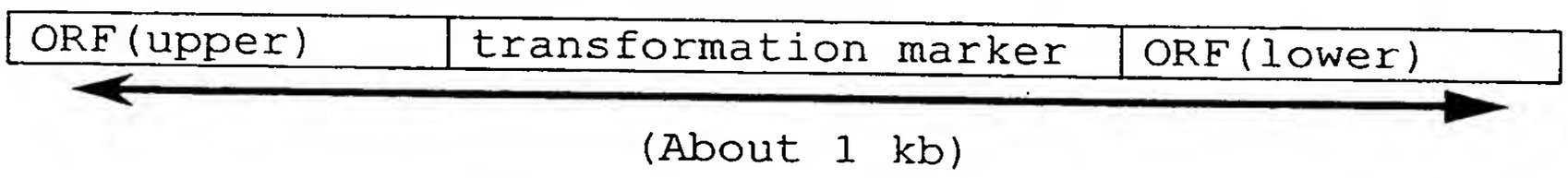
Method

1) Preparation of gene-disrupted strain

a-1) Preparation of gene-disrupted strain transformation cassette

In order to prepare a gene-disrupted strain transformation cassette, genes having chemical sensitivity; YPR036W(DEL000), YDL151C(DEL001), YGL026C(DEL003), YHR039C-A(DEL006), YKL080W(DEL007), YLR447C(DEL008), YGR006W(DEL012), YGR105W(DRL013), YJR104C(DEL014), YGL206C(DEL018), YIL036W(DEL019), YKL119C(DEL020), YLR226W(DEL022) and YLR311C(DEL025) in Table 7 were selected, and each gene was replaced with a transformation marker such as kanamycin resistance. As primers for performing PCT amplification, a N-terminal side (ORF(upper)) and a C-terminal side (ORF(lower)) in each ORF were used. A length of a sequence (ORF(upper) and ORF(lower)) of a primer homologues with ORF was 46 or 50 bp.

Gene gene-disrupted strain transformation cassette



Using these primers, and using a plasmid containing a gene sequence of a transformation marker as a template, a

PCR reaction was performed, and electrophoresis was preformed and, as a result, about 1 KD uniform bands were confirmed in primers for all genes. These PCR products were used as a gene-disrupted strain transformation cassette.

[0042]

a-2) Preparation and transformation of competent cell

As a strain from which a yeast gene-disrupted strain was prepared, W303 a mating-type ATCC200903 (MATα made2-1 trp1-1 leu2-3 leu2-112 his3-11 his3-15 ura3-1 can1-100) and W303 α mating type ATCC201238 (MATα ade2-1 trp1-1 leu2-3 leu2-112 his3-11 his3-15 ura3-1 can1-100) were used.

W303 a mating-type and W303α mating type competent cells were prepared and transformed with the previously prepared gene-disrupted strain transformation cassettes. For preparing and transforming competent cells, a commercially available kit (S.c. easyComp™ Transformation Kit: Invitrogen) was used.

[0043]

a-3) Confirmation of transformation

Transformation was confirmed using PCR. An upper primer was set in a promoter region of a targeting gene and a lower primer was set in a transformation marker, and PCR was performed. As a result, when an ORF site is replaced

with a transformation marker, and a gene is disrupted, a site between primers is amplified and, when a gene is not disrupted, the site is not amplified, thereby, transformation could be confirmed.

[0044]

b) Preparation of homozygous diploid and heterozygous diploid

By mixing-culturing haploids of Saccharomyces crevisiae a and α mating-types, an a/α-type diploid can be prepared.

A W303 a mating type (ATCC200903) and a W303α mating type (ATCC201238) in which the same gene was gene disruption-manipulated were mated by a mating procedure (Yeast Gene Experimental Manual: Maruzen Co., Ltd., p83-92) to prepare homozygous diploids. Separately, mating of a W303a mating type, and a non-gene-disrupted W303α type was performed by the similar procedure to prepare heterozygous diploids.

By such the procedure, homozygous diploids of DEL000, DEL001, DEL003, DEL006, DEL007, DEL008, DEL012, DEL013, DEL014, DEL018, DEL019, DEL020, DEL022 and DEL025 in Table 7 were prepared. In addition, heterozygous diploids in

which DEL006, DEL014 and DEL 022 were mated with a non-gene-disrupted strain (hereafter, referred to as DEL006 heterozygous diploid, DEL 014 heterozygous diploid, DEL022 heterozygous diploid) and, further, a heterozygous diploid in which DEL000 and DEL014 were mated (hereafter, referred to as DEL000/014 heterozygous diploid) were prepared.

[0045]

c) Preparation of promoter assay transformant

Competent cells of W303 ATCC201239 (MATa/MATα leu2-3/leu2-3 leu2-112/leu2-112 trp1-1/trp1-1 ura3-1/ura3-1 his3-11/his3-11 his3-15/his3-15 ade2-1/ade2-1 can1-100/can1-100) which is a parent strain of gene-disrupted strains, and each of prepared gene-disrupted strains were prepared. The competent cells were transformed using two kind of prepared plasmid for promoter assay, p-YBR072W (in which GFP is connected downstream of a promoter of YBR072W) and p-YPL171C (in which GFP is connected downstream of a promoter of YPL171C).

Specifically, p-YBR072W was prepared by the following procedure.

Primers for amplifying a polynucleotide (SCPD: disclosed in The Promoter Database of Saccharomyces cerevisiae) (SEQ ID No:1) containing a promoter sequence of

a yeast gene of YBR072W by PCR were prepared. Primers were designed using Oligo 4.0-S, Sequencher I, a McIntosh version, which is a software for designing primers, a nucleotide sequence of an upper primer is:

GCAGTCAACGAGGAGCGAATCAG (SEQ ID No: 2)

and a nucleotide sequence of a lower primer is:

GTTAATTTGTTTAGTTTGTTTG (SEQ ID No: 3)

In PCR, as a template, a yeast chromosome (Saccharomyces cerevisiae S288C, Cat.40802, Research Genetics, Inc.) was used and, as a reagent, a commercially available kit (KOD DNA Polymerase; code KOD-101, Toyobo) was used.

As a vector, pYES2(pYES2, Cat no:V825-20, Invitrogen Corporation, USA)(R.W.OLD, S.B. Primrose Principle of Gene Manipulation, Original Document, 5th Edition, Baifukan Co., Ltd., pp.234-263, 2000)) as a YEp-type shuttle vector which is replicated in both of Escherichia coli and yeast was used. As a polynucleotide encoding a marker protein, GFP, a part (SEQ ID NO: 4) of GFP of a vector pQBI 63 (Cat no.54-0082, Wako Pure Chemical Industries Ltd.) was used. First, a vector in which a polynucleotide of GFP was inserted into a multiple cloning site of pYES2 was prepared. Then, a part of a GAL promoter of pYES2 was replaced with a

polynucleotide containing a promoter sequence of YBR072W which is a yeast gene, to obtain an objective plasmid vector. A procedure of insertion of a polynucleotide containing GFP and a promoter sequence was performed by selecting appropriate restriction enzymes.

Then, a yeast strain or a gene-disrupted strain was transformed with this plasmid vector. A procedure of transformation is shown below.

1) A yeast cell, Saccharomyces cerevisiae W303, is shaking-cultured on 200 mL of a YPD medium until ODD660 becomes 0.5.

2) Cells are collected and suspended in 5 mL of a TE-buffer

3) 250 μL of 2.5 M lithium acetate is added.

4) Each 300 μL is dispended, and 10 μL of the plasmid vector is added, followed by culturing at 30°C and 30 minutes.

5) 700 μL of 50% PEG4000 is added, followed by shaking culturing at 30°C for 60 minutes.

6) After heat shock (42°C, 5minutes), the culture is rapidly cooled.

7) The culture is washed with 1 M sorbitol twice.

8) This is seeded on an agar plate made of a minimum nutrient medium (obtained by adding a necessary amino acid (adenine, histidine, tryptophan, leucine) to a SD medium).

Transformation was confirmed on a selective medium(SD medium (Yeast nitrogen base without amino acids (Difco 0919-15)+glucose+amino acid (adenine, histidine, tryptophan, leucine). Colonies which were grown on an agar plate of the selective medium were further confirmed for amino acid auxotrophy.

And, p-YPL171C was prepared as follows:

Primers for amplifying a polynucleotide (SCPD: disclosed in The Promoter Database of Saccharomyces cerevisiae)(SEQ ID No. 5) containing a promoter sequence of a yeast gene YPL171C by PCR was prepared. Primers were designed using Oligo 4.0-S, Sequencher I, a McIntosh version, which is a software for designing primers, a nucleotide sequence of an upper primer is:

ACGCCCCTTCCTTTTTCCCTTTC (SEQ ID No: 6)

and a nucleotide sequence of a lower primer is:

CTTCTAAATTTAAACTTCGCTA (SEQ ID No: 7)

In PCR, as a template, a yeast chromosome (Saccharomyces cerevisiae S288C, Cat.40802, Reserch Genetics, Inc.) was used and, as a reagent, a commercially available kit (KOD DNA Polymerase; code KOD-101, Toyobo) was used.

As a vector, pYES2 (pYES2, Cat no:V825-20, Invitrogen Corporation, USA) (R.W. Old, S.B. Primrose, Principle of Gene Manipulation, original document 5th edition, Baifukan Co., Ltd., pp.234-263, 2000) as a YEp-type shuttle vector which is replicated in both of Escherichia coli and yeast was used. In addition, as a polynucleotide encoding a marker protein GFP, a part (SEQ ID No: 4) of GFP of a vector pQBI 63 (Cat no.54-0082, Wako Pure Chemical Industries Ltd.) was used. First, a vector in which a polynucleotide of GFP was inserted into a multiple cloning site of pYES2 was prepared. Then, a part of a GAL1 promoter of pYES2 was replaced with a polynucleotide containing a prompter sequence of YPL171C which is a yeast gene, to obtain an objective plasmid vector. A procedure for inserting a polynucleotide containing GFP and a promoter sequence was performed by selecting appropriate restriction enzymes.

Then, a parent strain and a gene-disrupted strain were transformed with this plasmid vector. A procedure of transformation is shown below.

1) A yeast cell, Saccharomyces cerevisiae BY4743 is shaking-cultured on 200 mL of a YPD medium until OD660 becomes 0.5.

2) Cells are collected and suspended in 5 mL of a TE-buffer

3) 250 µL of 2.5 M lithium acetate is added.

4) Each 300 µL is dispended, and 10 µL of the plasmid vector is added, followed by culturing at 30°C for 30 minutes.

5) 700 µL of 50%PEG4000 is added, followed by shaking-culturing at 30°C for 60 minutes.

6) After heat shock (42°C, 5 minutes), the culture is rapidly cooled.

7) The culture is washed with 1 M sorbitol twice.

8) This is seeded on an agar plate made of a minimum nutrient medium (obtained by adding a necessary amino acid (histidine, leucine) to a SD medium).

[0046]

3) Chemical sensitivity test

The resulting transformant was grown to the steady state by shaking-culturing on a SD medium (adenine, hystidine, triptophan, leucine) at 25°C. The transformant in the steady state was diluted 500-fold with the same medium, shaking-cultured at 25°C for 15 hours and, after it was confirmed that an absorbance at 600 nm was 0.2 to 0.5 as a logarithmic growth phase, chemicals having different concentrations were loaded. After loading of chemicals, fluorescence of cells which had been cultured for 4 hours

was measured using a flow cytometer (FITC filter, EPICS XL-MCL, Bechmancoulter), and this was adopted as an expression amount of GFP (green fluorescence protein) which is a marker gene. A fluorescence intensity of 10000 cells was measured with a flow cytometer by one measurement and an average of fluorescence intensities of all cells was obtained, and was adopted as a measured value. Similarly, a fluorescence intensity of a cell to which a chemical had not been loaded was obtained, and results are shown as difference in a fluorescence intensity.

Results

Detection sensitivity of a promoter assay method when homozygous diploids of gene-disrupted strains DEL000, DEL001, DEL003, DEL006, DEL007, DEL008, DEL012, DEL013, DEL014, DEL018, DEL019, DEL020, DEL022 and DEL025 (Table 7) were used as a host cell was studied. Further, a detection sensitivity of a promoter assay method when heterozygous diploids of DEL006, DEL014 and DEL022 and a non-gene-disrupted strain, or a heterozygous diploid of DEL000 and DEL014 were used as a host cell, was studied. As a chemical to be loaded, sodium metaarsenite and thiuram exhibiting response when W303 was used as a host were selected and used in a promoter assay method using a plasmid p-YPL171C and benthiocarb was selected and used for

p-YER072W. A dilution series of a chemical was prepared and a loading test was performed. Results are shown in Fig. 6 to Fig. 11

[0047]

Fig. 6: DEL003, DEL006, DEL008, DEL014, DEL019 and DEL022 exhibited a fluorescent intensity equivalent to or more than that of a non-gene-disrupted stain by loading of a chemical at the same concentration. All gene-disrupted strains are a homozygous diploid.

Fig. 7: A DEL000 homozygous diploid, a DEL014 heterozygous diploid, a DEL 014 homozygous diploid and a DEL 000/014 heterozygous diploid exhibited a fluorescent intensity equivalent to or more than that of a non-gene-disrupted strain by loading of a chemical at the same concentration.

Fig. 8: DEL001, DEL006, DEL007, DEL018, DEL019, DEL020, DEL022 DEL025 exhibited a fluorescent intensity equivalent to or more than that of a non-gene-disrupted strain by loading a chemical at the same concentration. All gene-disrupted strains are a homozygous diploid.

Fig 9: A fluorescent intensity equivalent to or more than that of a non-gene-disrupted strain was exhibited by

loading of a chemical at the same concentration.

Fig 10: DEL006, DEL007, DEL012, DEL013, DEL020, DEL022 and DEL025 exhibited a fluorescent intensity equivalent to or more than that of a non-gene-disrupted strain by loading of a chemical at the same concentration. All gene-disrupted strains are a homozygous diploid.

Fig 11: A fluorescent intensity more than that of a non-gene-disrupted strain was exhibited by loading of a chemical at the same concentration.

Industrial Applicability

[0048]

From the results of a chemical sensitivity test with a chemical plate, gene-disrupted strains were selected and, actually, by using them as a host cell, chemical-responding gene recombinant cells were prepared, and chemical responsiveness was measured. As a result, about 1000-fold sensitivity was obtained in some chemicals. From this, it was confirmed that a host cell having necessary sensitivity for practical field may be developed by using this procedure.

In this time study of a host cell, gene-disrupted

strains exhibiting sensitivity to general chemicals were used, but possession of sensitivity to particular chemicals is considered to be advantageous in some cases, depending on a gene used in a reporter · gene · assay method and a targeting chemical.